

# Mutagenic Efficiency of Organophosphorus Insecticides Used in Combined Treatments

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Male mice (Q strain) received two consecutive injections of organophosphorus insecticides: a phosphonate (trichlorfon) was combined to a thiophosphate (methylparathion) or a dithiophosphate (malathion or methylazinphos) in order to evaluate the interactions at the genetic and cytogenetic levels.

No increase in chromosome damage was observed in bone marrow cells, spermatogonia, and primary spermatocytes. In a dominant lethal mutation assay, the frequency of postimplantation lethality was not significantly increased over the control level. The percentage of preimplantation losses was enhanced, probably due to a toxic effect on male germ cells.

## Introduction

The toxic effects induced by an organophosphorus insecticide can sometimes be modified by the action of another compound of the same group. Synergism occurs between trichlorfon and malathion or trichlorfon and methylazinphos. In contrast, there is an antagonism between malathion and parathion or malathion and methylazinphos (1,2). The potentiation of anticholinesterase action is explained by the inhibition of carboxylesterase and carboxamidase which detoxify the pesticides. The first compound competes with the detoxifying enzyme, impeding hydrolysis of the other, which results in a protracted action (3). These properties explain the increasing number of complex commercial insecticides which contain more than one active substance. The purpose of such associations is to produce both a simultaneous control of several pests and a toxicity potentiation.

On the other hand, some organophosphorus pesticides can induce mutations in microorganisms but most of them are generally ineffective in mammals treated *in vivo* (4).

The purpose of the present study is to evaluate the possibility of synergisms for cytogenetic (chromosome damage in bone marrow cells, spermatogonia and primary spermatocytes) and genetic (dominant lethal mutations) effects induced in mice after acute *in vivo* treatments.

Treatments were performed, combining phosphonate (trichlorfon) and a thiophosphate (methylparathion) or a dithiophosphate (malathion or methylazinphos). The results obtained with these four compounds tested separately have been reported elsewhere (5-8).

## Materials and Methods

The four organophosphorus compounds, trichlorfon (dimethyl 1-hydroxy-2,2,2-trichloroethyl phosphonate), methylparathion (dimethyl *p*-nitrophenyl phosphorothionate), malathion [dimethyl S-(1,2 diethoxycarbonyl) phosphorothionothiolate], and methylazinphos [dimethyl S-(4-oxobenzotriazino-3-methyl) phosphorothionothiolate] were Riedel-De Haen products, guaranteed 99% pure (Pestanal). Solutions are prepared extemporaneously.

In combined treatments, the two compounds were injected consecutively. Control and treated groups were tested concurrently. The insecticides were tested at the highest tolerated doses (trichlorfon, 100 mg/kg; methylparathion, 10 mg/kg; malathion, 300 mg/kg; methylazinphos, 1 mg/kg), or were decreased by 50% in combined treatments.

For the genetic and cytogenetic assays, 25 three- to four-month old male mice (Q strain) in each treated group received IP injections. Twenty untreated males were used as a negative control.

Cytogenetic effects in bone marrow cells were analyzed for two animals selected using a randomization table and sacrificed 12, 24, and 36 hr after treatment. The same schedule was followed for spermatogonia.

After a 10- to 12-day recovery period which corres-

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ponded to the treatment of A4-B type spermatogonia, two males were randomly selected on a daily basis and were sacrificed for the detection of chromosome aberrations in primary spermatocytes at diakinesis-metaphase I.

Microscopic slides were prepared according to the air drying method, were stained with Giemsa and permanently mounted. Slides (5/animal) were coded prior to microscopic analysis. Only well spread metaphases (maximum of 50 bone marrow cells, 50 spermatogonia or 100 spermatocytes per slide) were taken into consideration.

Five males were selected for the dominant lethal assay. They were each mated with four untreated virgin females which were replaced weekly for seven consecutive weeks. Pregnant females were killed 14 days after the detection of vaginal plugs. The number of corpora lutea was counted for each ovary. The number of live embryos, deciduomas and late fetal deaths was determined for each uterine horn. Fetal lethality is expressed

as a percentage of the corpora lutea (preimplantation losses and total mortality), or of implants (postimplantation losses). Each positive result was confirmed by a second independent experiment using twice the number of animals.

Statistical analysis was carried out using the Kastenbaum and Bowman tables and the  $\chi^2$  test.

## Results and Discussion

### Toxic Effects

A preliminary experiment was conducted to select the appropriate doses for the cytogenetic and genetic assays. As compared to the single treatments, a significant enhancement of the toxicity was obtained for the combined treatments (Table 1). Nevertheless, it seems difficult to conclude that a real synergism of

Table 1. Toxic effects induced by trichlorfon alone and by combined treatments.

Treatment	Mortality	
	No.	%
Trichlorfon, 100 mg/kg	0/59	0.0
Methylparathion, 10 mg/kg	5/71	7.0
Trichlorfon, 100 mg/kg + methylparathion, 10 mg/kg	6/10	60.0
Trichlorfon, 50 mg/kg + methylparathion, 5 mg/kg	1/78	1.3
Malathion, 300 mg/kg	0/58	0.0
Trichlorfon, 100 mg/kg + malathion, 300 mg/kg	9/10	90.0
Trichlorfon, 50 mg/kg + malathion, 150 mg/kg	4/73	5.5
Methylazaphos, 1 mg/kg	0/56	0.0
Trichlorfon, 100 mg/kg + methylazaphos, 1 mg/kg	7/20	35.0
Trichlorfon, 50 mg/kg + methylazaphos, 0.5 mg/kg	0/77	0.0

Table 2. Cytogenetic effects induced in bone marrow cells by trichlorfon alone and by combined treatments (500 metaphases analyzed).

Treatment	Recovery period, hr	Percentage of aberrations		
		Breaks	Exchanges	Gaps
Control	—	0.4	0.0	0.4
Trichlorfon, 100 mg/kg	12	0.6	0.0	0.6
	24	0.6	0.2	0.6
	36	0.0	0.0	0.4
Trichlorfon, 50 mg/kg + methylparathion, 5 mg/kg	12	0.8	0.0	0.0
	24	0.0	0.0	0.2
	36	0.6	0.0	0.0
Trichlorfon, 50 mg/kg + malathion, 150 mg/kg	12	0.2	0.0	0.0
	24	0.0	0.0	0.0
	36	0.4	0.0	0.0
Trichlorfon, 50 mg/kg + methylazaphos, 0.5 mg/kg	12	0.0	0.0	0.0
	24	0.2	0.0	0.6
	36	0.0	0.0	0.0

Table 3. Cytogenetic effects induced in spermatogonia by trichlorfon alone and by combined treatments.

Treatment	Recovery period, hr	Number of metaphases analyzed	Aberrations					
			Breaks		Exchanges		Gaps	
			No.	%	No.	%	No.	%
Control	—	428	2	0.47	0	0.00	1	0.23
Trichlorfon, 100 mg/kg	12	317	3	0.95	0	0.00	2	0.63
	24	252	0	0.00	0	0.00	2	0.79
	36	346	1	0.29	0	0.00	1	0.29
Trichlorfon, 50 mg/kg + methylparathion, 5 mg/kg	12	345	0	0.00	0	0.00	1	0.29
	24	442	1	0.23	0	0.00	1	0.23
	36	469	2	0.43	0	0.00	0	0.00
Trichlorfon, 50 mg/kg + malathion, 150 mg/kg	12	326	1	0.31	0	0.00	1	0.31
	24	344	1	0.29	0	0.00	0	0.00
	36	360	0	0.00	0	0.00	0	0.00
Trichlorfon, 50 mg/kg + methylazaphos, 0.5 mg/kg	12	274	1	0.36	0	0.00	0	0.00
	24	262	0	0.00	0	0.00	0	0.00
	36	194	0	0.00	0	0.00	0	0.00

action took place. During the acute toxicity experiments, we observed that the maximum tolerated dose was generally close to the 100% lethal dose. Therefore it was evident that a double dose could induce more than a

double lethality. On the other hand, the combined treatment with 50% decreased doses was only slightly toxic. Our results probably express more of an additive effect than a real synergism.

**Table 4. Cytogenetic effects induced at primary spermatocytes by trichlorfon alone and by combined treatments (1000 metaphases analyzed).**

Treatment	Recovery period, days	Percentage of aberrations		
		Breaks	Exchanges	Gaps
Control	—	0.3	0.0	0.0
Trichlorfon, 100 mg/kg	10	0.3	0.1	0.2
	11	0.4	0.0	0.2
	12	0.6	0.0	0.0
Trichlorfon, 50 mg/kg + methylparathion, 5 mg/kg	10	0.1	0.0	0.0
	11	0.3	0.0	0.1
	12	0.0	0.0	0.0
Trichlorfon, 50 mg/kg + malathion, 150 mg/kg	10	0.5	0.0	0.0
	11	0.5	0.0	0.0
	12	0.5	0.0	0.1
Trichlorfon, 50 mg/kg + methylazinos, 0.5 mg/kg	10	0.2	0.0	0.0
	11	0.4	0.0	0.1
	12	0.2	0.0	0.0

## Cytogenetic Effects

Due to the increased toxicity, the combined treatments were performed with the half-maximum tolerated dose. The number of lesions induced by the organophosphorus compounds was too small to consider individually as being aberrations of the chromatid and chromosome types.

The frequency of chromosome aberrations in bone marrow metaphases was not higher in treated males than in untreated controls (Table 2). In spermatogonia (Table 3), the percentage of chromosome lesions was higher after trichlorfon single treatment than after the combined treatments.

The same conclusion was reached for diakinesis (Table 4). For the three test systems, we did not observe a statistically significant difference between the treated groups and the negative control group, or between the combined treatment and the single trichlorfon treatment.

**Table 5. Genetic effects induced by trichlorfon alone and by combined treatments.**

Treatment	Recovery period, days	No. of pregnant females	Corpora lutea per ♀	Implants per ♀	Preimplantation losses		Live embryos per ♀	Postimplantation losses		Total mortality	
					per ♀	% CL		per ♀	% Imp.	per ♀	% CL
Control	—	68	9.15	9.93	0.78	7.9	8.78	0.37	4.0	1.15	11.6
Trichlorfon, 100 mg/kg	1-7	11	10.18	9.36	0.82	8.0	9.18	0.18	1.9	1.00	9.8
	8-14	11	9.73	8.46	1.27	13.1	8.37	0.09	1.1	1.36	14.0
	15-21	40 <sup>a</sup>	9.88	9.46	0.42	4.3	8.98	0.48	5.0	0.90	9.1
	22-28	34 <sup>a</sup>	9.56	8.15	1.41	14.8*	7.88	0.27	3.2	1.68	17.5
	29-35	40 <sup>a</sup>	10.35	9.22	1.13	10.9	8.97	0.25	2.7	1.38	13.3
	36-42	14	9.00	7.93	1.07	11.9	7.36	0.57	7.2	1.64	18.2
	43-49	14	10.50	9.43	1.07	10.2	9.36	0.07	0.8	1.14	10.9
Trichlorfon, 50 mg/kg + methylparathion, 5 mg/kg	1-7	13	9.77	8.69	1.08	11.0	8.15	0.54	6.2	1.62	16.5
	8-14	14	10.64	9.00	1.64	15.4*	8.64	0.36	4.0	2.00	18.8
	15-21	13	10.77	9.46	1.31	12.1	8.85	0.61	6.5	1.92	17.9
	22-28	9	11.00	10.22	0.78	7.1	10.00	0.22	2.2	1.00	9.1
	29-35	40 <sup>a</sup>	10.08	8.60	1.48	14.6*	8.43	0.17	2.1	1.65	16.4
	36-42	31 <sup>a</sup>	10.29	8.84	1.45	14.1*	8.35	0.48	5.5	1.84	18.8
	43-49	44 <sup>a</sup>	10.36	8.66	1.70	16.4*	8.32	0.34	3.9	2.05	19.7
Trichlorfon, 50 mg/kg + malathion, + 150 mg/kg	1-7	41 <sup>a</sup>	10.41	8.44	1.98	19.0*	8.17	0.27	3.2	2.24	21.6
	8-14	41 <sup>a</sup>	10.24	8.49	1.76	17.1*	8.24	0.24	2.9	2.00	19.5
	15-21	17	10.24	9.59	0.65	6.3	8.76	0.83	8.6	1.47	14.4
	22-28	17	9.94	8.65	1.29	13.0	8.41	0.24	2.7	1.53	15.4
	29-35	42 <sup>a</sup>	10.55	8.83	1.71	16.3*	8.55	0.29	3.2	2.00	19.0
	36-42	16	10.44	9.50	0.94	9.0	9.13	0.37	3.9	1.31	12.6
	43-49	39 <sup>a</sup>	9.79	8.28	1.51	15.5*	7.69	0.59	7.1	2.10	21.5
Trichlorfon, 50 mg/kg + methylazinos, 0.5 mg/kg	1-7	13	11.08	9.85	1.23	11.1	9.31	0.54	5.5	1.77	16.0
	8-14	17	10.82	9.82	1.00	9.2	9.59	0.24	2.4	1.24	11.4
	15-21	20	10.15	9.00	1.15	11.3	8.60	0.40	4.4	1.55	15.3
	22-28	47 <sup>a</sup>	10.45	8.60	1.85	17.7*	8.28	0.32	3.7	2.17	20.8
	29-35	19	10.37	9.16	1.21	11.7	8.89	0.27	2.9	1.48	14.2
	36-42	18	10.22	9.11	1.11	10.9	8.89	0.22	2.4	1.33	13.0
	43-49	17	10.12	9.24	0.88	8.7	8.82	0.41	4.5	1.29	12.8

<sup>a</sup>Data from two independent experiments.

\* $p < 0.01$ .

## Genetic Effects

The treatments did not induce male sterility and the percentage of pregnant females was not affected. The matings performed after combined trichlorfon-methylparathion treatments gave a frequency of deciduomata not significantly increased over the control level; the number of live embryos per litter was always higher than 8 (Table 5). At the same time, the preimplantation lethality was important and averaged 1.46 losses per female for the entire assay. Almost similar conclusions could be drawn after trichlorfon-malathion combined treatments. The postimplantation mortality was not significantly enhanced, the number of live embryos was normal but the preimplantation lethality was doubled as compared to the control group (average of 1.56 losses per female for the entire assay).

Except for the fourth mating week, the results obtained after the trichlorfon-methylazinhos combined treatments were negative. The only positive data concerned once again an increase in the preimplantation lethality without significant increase in the postimplantation mortality or decrease in the size of the litter.

However, when the results obtained with combined treatments are compared to those observed for trichlorfon treatment, only the preimplantation lethality induced during the first and fifth weeks after the trichlorfon-malathion combined treatment increase significantly.

## Discussion

It is of course difficult to compare the effects of a single treatment of the maximum tolerated dose with those obtained after combined treatments at 50% decreased doses.

Cytogenetic assays gave only negative results confirming those observed with the four single compounds (5-8). No interaction was observed in these test systems.

In the dominant lethal mutation assay, no increase of the postimplantation fetal mortality occurred, and we could not determine a mutagenic effect. Nevertheless, the preimplantation losses were widely enhanced over the control level.

But when compared to the single trichlorfon treatment, only the trichlorfon-malathion treatment produced positive results. Since preimplantation lethality could be correlated to a toxic effect on male germ cells, this data confirms the increase in toxicity observed for the preliminary toxicity test.

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